

Metabolic rates of groundwater species as a function of body mass and temperature

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Abstract

Research on the metabolic physiology of groundwater species, particularly regarding oxygen consumption rates (OCR), has made significant advancement, revealing valuable insights into the adaptations of exclusively groundwater-dwelling (stygobitic) species. However, a comprehensive understanding of how these metabolic rates scale with body mass and respond to temperature changes remains elusive. This study aims to bridge this gap by reviewing published data on OCR across a variety of groundwater organisms to elucidate patterns of metabolic rates in relation to body size and temperature. We employed a combination of literature review and quantitative analyses, focusing on the allometric scaling of OCR with body weight and the effect of temperature on metabolic rates. Our findings indicate that OCR scales with body weight in an allometric pattern, with an inter-species slope of 0.80, suggesting non-isometric scaling. Furthermore, our analysis showed that stygobitic species' metabolic rates are less responsive to warming than those of non-stygobitic species at low to moderate temperatures. However, at higher temperatures, metabolic rates in stygobitic species decline faster than in non-stygobitic taxa, highlighting a potential vulnerability to global climate change. This study contributes to our understanding of the metabolic strategies of groundwater species, underscoring the need for further research to fully grasp the eco-evolutionary implications of these findings for groundwater conservation.

Keywords

Allometry, Arrhenius equation, ectotherms, metabolism, oxygen consumption rate, respiration, stygobiont

Introduction

Metabolism encompasses the entirety of biochemical reactions within an organism, essential for sustaining cellular functions and the whole biosphere (Braakman and Smith 2013). This process, which involves deriving energy from fuel molecules, is inherently inefficient: a portion of the energy is invariably converted into heat, a form not practically utilizable (Balaban 2020). Endotherms, such as mammals and birds, harness and regulate metabolic heat to maintain stable internal body temperatures and peak sustained performance (Ruben 1995). In contrast, ectotherms, including invertebrates, reptiles, amphibians, and fish, do not rely on metabolic heat for temperature regulation, leading to internal temperatures that fluctuate with environmental changes (Angilletta 2009). The metabolic rate is the energy an organism consumes over a specific period of time, quantifiable through calorimetry by measuring an organism's heat loss. This measurement can be expressed in joules (J), calories (cal), or kilocalories (kcal) per unit of time (Kaiyala and Ramsay 2011). Alternatively, it can be assessed through respirometry, which calculates the oxygen consumption rate as the volume of oxygen consumed over time (Lampert 1984). Oxygen consumption rates (OCR) vary widely across taxa, and can be influenced by factors such as organism's body mass (Gillooly et al. 2001), environmental conditions (e.g., temperature; Hernández-León and Ikeda 2005) and activity level (Culver and Poulson 1971). Standard OCR are a proxy of standard metabolic rates (SMR), which refer to the metabolic rates measured under conditions of rest, tranquility, absence of stress, and fasting (Angilletta 2009). Typically, SMR scale with body mass in both endothermic and ectothermic species (Gillooly et al. 2001). This relationship is represented by the allometric law (West et al. 1997) in equation 1:

$$SMR = SMR_0 \times M^b \quad (1)$$

Here, SMR_0 is a constant that is characteristic of the kind of organism, M is the organism's body mass, and b is the scaling exponent dictating the relationship's slope. The equation 1 can be conveniently log-transformed as in equation 2:

$$\log(SMR) = \log SMR_0 + b \log M \quad (2)$$

where: $b > 1$ indicates that SMR increase at a faster rate than mass; $b < 1$ indicates that SMR increase at a slower rate than mass and $b = 1$ indicates isometry, i.e. SMR scale proportionally with mass. For organisms to stay in energy balance, metabolism can only vary in proportion to their surface area (Rubner 1883). Kleiber (1932) experimentally found a (close to) $3/4$ exponent to describe the relationship between metabolic rate and body mass. Notably, many invertebrate taxa exhibit a mass exponent b in the range of 0.66–0.80, a shared characteristic whose underlying reason remains elusive (Hoppeler and Weibel 2005).

Temperature has profound effects on chemical and biochemical reactions and aerobic metabolism at the cellular level, thereby shaping the metabolic rates of ectotherms

(Schulte et al. 2015). Within permissive thermal ranges, which are temperatures conducive to long-term survival, an increase of +10 °C often leads to a doubling or tripling of metabolic rates (Dell et al. 2011). This effect can be quantified using Q_{10} (the factorial change in metabolic rates resulting from a 10 °C increase) or by E_a (Arrhenius activation energy; Cossins and Bowler 1987). For most ectotherms, E_a typically ranges from 0.5 to 0.8 eV (= 48.26 to 77.22 kJ/mol; $Q_{10} = 2$ or 3), equating to a 7–12% increase in metabolic rates per degree Celsius (Dell et al. 2011). Initially, in ectotherms, warming positively affects physiological processes like developmental speed, mobility and egg production (MacLean et al. 2019). However, excessive warming accelerates aging and senescence, and beyond a critical temperature threshold, heat-related failure can occur rapidly, with severe consequences (Brown et al. 2004). The physiological causes of heat failure in ectotherms, potentially including protein denaturation, oxygen limitation, cellular excitability loss and membrane dysfunction, are not fully understood (Brown et al. 2004). The severity of temperature stress depends on both the temperature and duration of exposure, with thermal tolerance limits significantly influencing species distributions (Rezende et al. 2014). According to the metabolic theory of ecology, metabolic rate varies with body mass and temperature as a result of internal physical constraints (Glazier et al. 2020). However, various ecological factors may also affect metabolic rate and its scaling with body mass. Glazier et al. (2020), for example, have shown that the effect of temperature on the ontogenetic scaling of resting metabolic rate of the freshwater amphipod *Gammarus minus* depends critically on habitat differences in predation regime.

Groundwater habitats, typically oligotrophic and devoid of light, feature stable temperatures and chemical conditions, which reduce the need for rapid physiological adaptations (Di Lorenzo et al. 2023). Stygobitic species (i.e., species that are unable to complete their life cycle outside of groundwater habitats; Culver et al. 2023), have been shown to generally exhibit lower metabolic rates than their surface water counterparts (Hüppop 1986; Hervant et al. 1997; Di Lorenzo et al. 2015). However, a few studies have demonstrated the opposite pattern. Simčič and Sket (2019) observed that the OCR did not differ significantly between species or subspecies of the same genus from surface and subterranean habitats, but still they responded differently to temperature changes. Undoubtedly, our current understanding of metabolic variations across groundwater metazoans and their surface water relatives is limited. A comprehensive review of the OCR of stygobitic species is still lacking. Additionally, the influence of environmental factors, like temperature, on OCR in stygobitic species and their compliance with allometric scaling principles represent significantly underexplored areas. Gaining a better understanding of the physiological performance of subterranean organisms is of paramount importance, especially in the face of climate change (Vacca-relli et al. 2023). The persistence of species with poor dispersal abilities and high habitat specialization, such as groundwater species, will strongly rely on their physiological capacity to cope with environmental changes (Pallarés et al. 2020).

We aim to address the knowledge gaps in understanding the patterns of metabolic rates in groundwater species by reviewing the published data reporting on OCR of these organisms. The specific objectives are testing, for groundwater organisms,

the validity of existing models on metabolic rates' dependencies on a) body size and b) temperature. Since the whole-body metabolic rate is central to the understanding of physiological as well as of ecological function of species, this review endeavors to enhance our understanding of the metabolic physiology of groundwater organisms, offering insights for their effective conservation and management efforts.

Material and methods

Data collection

We systematically searched and collected data from both peer-reviewed and grey literature sources. We conducted the literature search using the Web of Science platform, applying a set of pre-defined criteria. Our selection criteria were specifically designed to target studies quantifying metabolic rates in groundwater metazoans by measuring OCR. Following preliminary investigations in December 2023, various search terms were trialed, leading to the consensus adoption of the search string: TS = ("oxygen consumption rate*" OR "metabolic rate*" OR metabolism) AND TS = (groundwater OR stygob* OR subterranean). The initial search in December 2023 yielded 2,236 papers. We meticulously examined the complete content of each identified article and thoroughly scrutinized the reference lists within these articles to identify any supplementary pertinent sources. Additionally, our research approach extended beyond Web of Science, encompassing an unstandardized exploration of gray literature sources. Half of the studies retrieved from the literature focused on microbiology, engineering, agronomy, and other topics not pertinent to environmental science and ecology. Of the remaining studies, 14% were on terrestrial subterranean species, 10% on marine species, and 24% lacked quantitative OCR measurements (e.g., studies on metabolites). Following the screening phase, we identified 23 papers. In the next step, we meticulously reviewed the full text of each paper and extracted various key data points, including the type of publication, year of study, geographic and taxonomic scope, type of groundwater habitat, life history traits, morphometric data, field observations, acclimation data, and measurements of OCR. Additionally, we extracted data for non-stygobitic species that were examined in some of the papers for comparative purposes. Whenever the results of OCR measurements were not reported in tables or in text, we employed a web-based plot digitizer tool (<https://automeris.io/WebPlotDigitizer>) to extract OCR data from plots and images when the data were presented only graphically.

We obtained a total 291 OCR measurements from 23 studies (see the Results section for more details). To address our research questions on metabolic scaling and the impact of temperature on metabolic rates, we created two sub-datasets. For detailed information on these datasets, please refer to the relevant paragraphs in the subsequent sections. We converted all obtained data on OCR to a standard unit of $\mu\text{L O}_2/\text{mg DW}/\text{hour}$ (DW = dry weight). In cases where dry weight was not reported, we estimated it to be 15% of the wet mass, based on the methodology used by Wilhelm et al. (2006)

for two amphipod species, which was in turn based on Taylor et al. (2003). This conversion was applied because approximately 70% of the records with indicated wet weight pertained to amphipod species. Oxygen mass (in $\mu\text{g O}_2$) and oxygen in moles ($\mu\text{mol O}_2$) were converted into volume ($\mu\text{L O}_2$) using a conversion factor of 1.43 (Gnaiger 1983; Peters 1983) and 22.4 (Lampert 1984), respectively. We applied no temperature corrections, as we recorded the temperatures at which each study was conducted.

Metabolic scaling

To investigate the relationship between body weight and OCR, we compiled a data set of 10 stygobitic species and 64 measurements. We collected data on 2 copepod species [*Diacyclops belgicus* Kiefer, 1936 and *Moraria* sp.], 3 isopod species [*Proasellus lusitanicus* (Frade, 1938); *Stenasellus virei* Dollfus, 1897; *Asellus aquaticus cavernicolus* Racovitza, 1925], 4 amphipod species [*Niphargus rhenorhodanensis* Schellenberg, 1937; *Niphargus virei* Chevreux, 1896; *Niphargus krameri* Schellenberg, 1935; *Niphargus stygius* (Schiödt, 1847)], and one amphibian species [*Proteus anguinus* Laurenti, 1768]. We included data only from studies that measured adult specimens at rest that had fasted for no more than 15 days prior to measurements and were not exposed to additional stressors (e.g., temperature stress, anoxia). We excluded studies where the experimental temperatures exceeded the species' permissive ranges, thereby ensuring data at temperatures consistent with their native habitats. Some authors referred to the OCR of resting, fasted animals without additional stressors as “standard metabolic rates” (e.g., Di Lorenzo and Galassi 2017), while others termed them “routine metabolic rates” (e.g., Simčič and Sket 2019). This variation in terminology probably stems from differences among species in their levels of random activity during respirometry trials. However, previous research has shown no discernible effect of author classification on metabolic scaling with body weight for other taxa (Killen et al. 2010; Boyce et al. 2020). We considered the specified OCR values in this study as the most appropriate proxies for standard metabolic rates, defined as metabolic rates measured under conditions of rest, tranquility, absence of stress, and fasting (Angilletta 2009). To prevent bias from multiple measurements per species, we included only one OCR value per species per study, as suggested by Clarke and Johnston (1999). To obtain one OCR value per species, when multiple data points existed for a species within a study, we used the median values to minimize the impact of potential outliers.

We used a linear model to examine the expected allometric relationship between species DW (in mg) and OCR (in $\mu\text{L O}_2/\text{h} \times \text{ind.}$). We log-transformed OCR and DW values. We assessed the normality of the residuals using both a Shapiro-Wilk test and visual inspection of QQ-plot (Suppl. material 1: fig. S1).

Temperature effects

We compiled data from the literature for 19 stygobitic species and 288 measurements to assess the dependence of metabolic rate estimated from OCR on temperature. Collected data included 2 copepod species [*D. belgicus* and *Moraria* sp.], 6 isopod

species [*P. lusitanicus*; *Proasellus valdensis* (Chappuis, 1948); *Proasellus* sp. 1 (gr. *cavaticus*); *Proasellus* sp. 2 (gr. *cavaticus*); *S. virei*; *A. aquaticus cavernicolus*], 7 amphipod species [*N. rhenorhodanensis*; *N. virei*; *N. krameri*; *N. stygius*; *Stygobromus* sp.; *Stygobromus pecki* (Holsinger, 1967); *Gammarus acherondytes* Hubricht & Mackins, 1940)], 2 decapod species [*Procambarus franzi* Hobbs & Lee, 1976, *Procambarus pallidus* (Hobbs, 1940)], one amphibian species [*P. anguinus*] and a fish species [*Astyanax mexicanus* (De Filippi, 1853)]. Aquatic subterranean organisms must cope with periodic oxygen deficiency in their habitats, which can sometimes involve rapid changes from normoxia (> 3 mg/L O_2) to hypoxia (in the range of 0.3 to 3 mg/L O_2) or even anoxia (< 0.3 mg/L O_2 ; Malard and Hervant 1999). Previous studies have demonstrated that they are resistant to hypoxia, although they cannot survive severe hypoxia (dissolved oxygen < 0.01 mg/L O_2) for more than two days (Malard and Hervant 1999). Accordingly, the OCR values in our dataset were quite comparable in normoxia and hypoxia for some species. For this reason we included data for OCR measured in both normoxic and hypoxic conditions. This approach allowed us to capture the physiological responses of these organisms to the varying oxygen levels they naturally experience. As in the metabolic scaling analyses, we analysed all data collectively, without differentiation into standard or routine categories. We included in the calculation the OCR (in $\mu\text{L } O_2/\text{mg DW} \times \text{h}$) and the incubation temperatures at which the animals were acclimatised for at least 5 days prior measurements. For an illustrative presentation of compiled data regarding the dependence of metabolic rate on temperature, we divided all OCR into 11 temperature classes with an interval of 3 degrees, from -2°C to 30°C , and calculated mean and standard deviation (SD) for the stygobitic and non-stygobitic groups of species (Suppl. material 1: table S1).

The thermodynamic response of the OCR over the entire temperature range was calculated in terms of the Arrhenius activation energy (E_a), which describes the influence of temperature on the metabolic rate. An estimate of E_a was derived from the slope of the Arrhenius plot of the natural logarithm of the OCR against the reciprocal of the absolute temperature (Robinson and Williams 1993) according to the equation 3:

$$E_a = -R_{gc} S \quad (3)$$

where E_a is expressed in kJ/ mol, R_{gc} is the gas constant ($8.314 \text{ J/ mol} \times \text{K}$), and S is the slope of the Arrhenius plot. We calculated the slope separately for low (from -2°C to 18°C) and high (from 19°C to 30°C) temperature ranges, as the mean OCR increases up to the $16\text{--}18^\circ\text{C}$ temperature class and after that decreases with temperature (Suppl. material 1: table S1). For comparative analysis, we conducted the same assessments of the relationship between temperature and metabolic rates using comparable data obtained from 12 non-stygobitic species concurrently sourced from the same literature. We compiled data from the literature for: 1 copepod species [*Eucyclops serrulatus* (Fischer, 1851)], 4 amphipod species [*Gammarus fossarum* Koch, 1836; *Gammarus lacustris* G.O. Sars, 1863; *Synurella* sp.; *Niphargus zagrebensis* S.

Karaman, 1950], 2 isopod species [*Asellus aquaticus aquaticus* Linnaeus, 1758; *Asellus aquaticus carniolicus* Sket, 1965], 2 decapod species [*Procambarus pictus* (Hobbs, 1940), *Procambarus clarkii* (Girard, 1852)], 1 cyprinodontiformes [*Poecilia mexicana* Steindachner, 1863], 1 Cypriniformes [*Gobio occitaniae* Kottelat & Persat, 2005] and 1 Characiformes [*Psalidodon fasciatus* (Cuvier, 1819)]. Since most of the data come from studies in which OCR was measured at only one or two temperatures, we pooled the data of all stygobitic or non-stygobitic species into two groups to assess the general trend in the relationship between OCR and temperature for stygobionts and compare it with the relationship for non-stygobionts, as data sets of both groups were obtained using the same approach. We compared the slopes of the Arrhenius plots between stygobitic and non-stygobitic species using ANCOVA to ascertain if there were significant differences in the E_a of the two groups. We used a Shapiro-Wilk test to assess the normal distribution of the data, and a Levene's test to examine the equality of variances. We performed all statistical analyses using SPSS 20.0 (SPSS Inc., Chicago, Illinois, USA).

Results

We collected 23 papers originating from Nearctic (Canada, USA, Mexico), and Palearctic Regions (France, Portugal, Spain, Italy, Slovenia) (Fig. 1). Publication years spanned from 1971 to 2023, showing a notable uptick starting in 2000 (Fig. 1). We gathered 291 OCR measurements across five taxonomic classes, encompassing 37 species (Table 1), of which 23 were stygobitic (186 measurements) and 14 non-stygobitic (105 measurements). Within the stygobitic taxa, the order Amphipoda was the most studied with 11 species and 106 OCR measurements, followed by the order Isopoda, with 7 species and 54 measurements (Fig. 1). Studies investigating the impact of temperature on the OCR of stygobitic taxa focused on the orders Harpacticoida, Cyclopoida, Isopoda, and Amphipoda (Fig. 1). However, only amphipods were examined regarding the effects of both warming and cooling (Fig. 1). The stygobitic species examined in this study were primarily collected from caves, followed by karst springs and alluvial aquifers, with no hyporheic species included (Fig. 1). The 291 records of OCR, also including type of groundwater habitat, life history traits, morphometric data, field observations, acclimation data, and measurements of OCR is available in Figshare (<https://doi.org/10.6084/m9.figshare.25564377.v4>) in Excel (.xlsx) format with read.me text file describing metadata. We reported the average OCR per species in Table 1. Dry weight ranged from 0.001 to 3185 mg (Table 1).

For 25 out of the 37 species in this study, the collection site temperature was below or equal to 18 °C (Table 1). Mean OCR, measured in $\mu\text{L O}_2/\text{h} \times \text{mg DW}$, varied among species, ranging from 0.043 (*Astyanax mexicanus*) to 6.7 (*D. belgicus*) for stygobitic species, and from 0.13 (*Gobio occitaniae*) to 14.86 (*Gammarus lacustris*) for non-stygobitic species (Table 1). In the analyzed data set, non-stygobitic species demonstrated higher

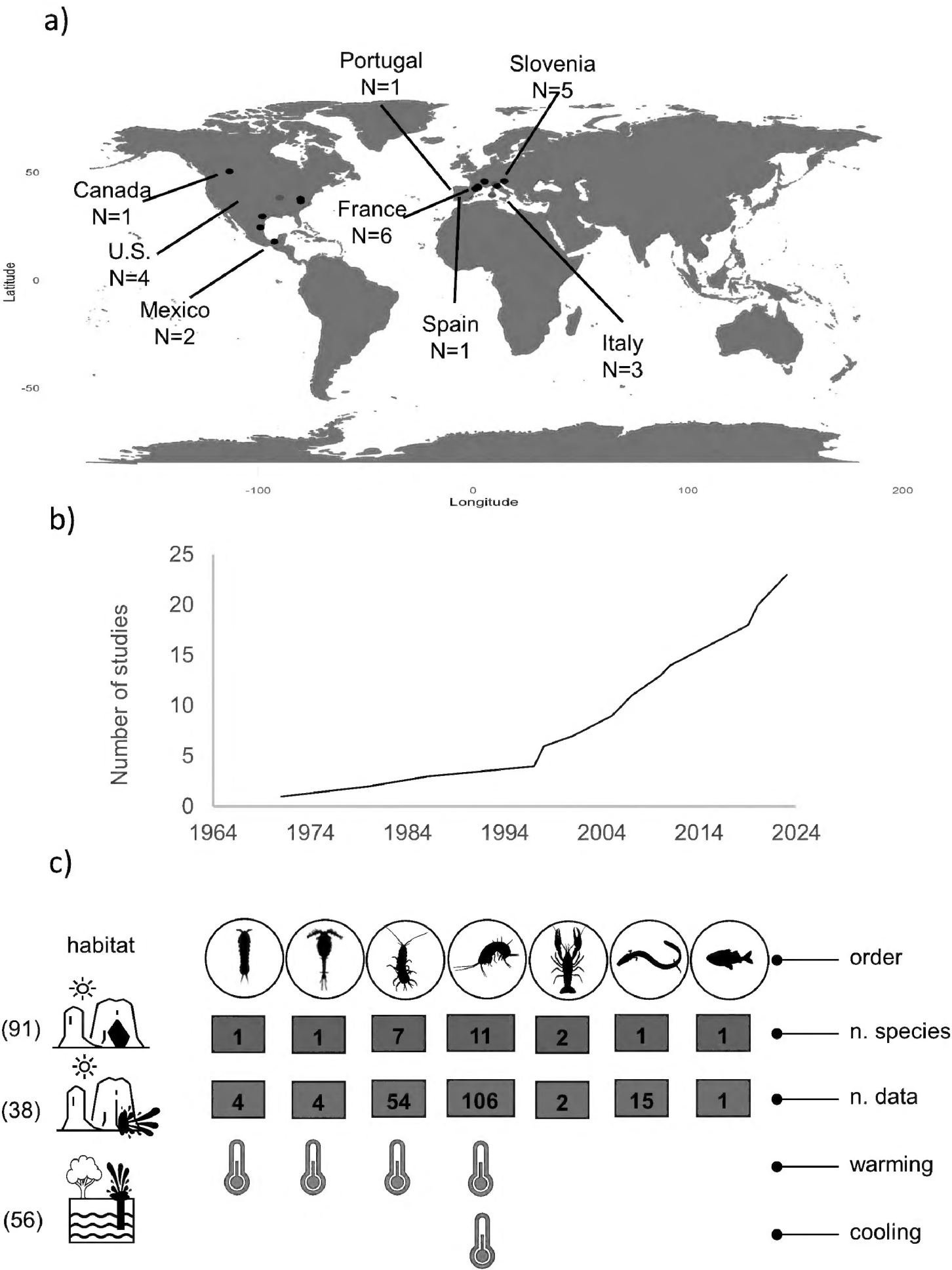


Figure 1. Geographic distribution of the 23 studies included in our dataset (a), with the timeline of publication years (b). N indicates the number of studies per country. The lower portion of the panel shows the distribution of the 186 OCR (oxygen consumption rate) measurements derived from these studies for stygobitic species (c). Icons provide a breakdown of the number of species per taxonomic order, and details on studies that examined the effects of warming and cooling. Information on the number of OCR measurements categorized by habitat type is also depicted (caves, springs and alluvial aquifers).

Table 1. Mean values of oxygen consumption rates per taxon. E = ecology (SB: stygobitic; nSB: non-stygobitic; NA- not well defined); DW = mean dry weight (mg); T (temperature of the collection site; °C); OCR (mean oxygen consumption rates in $\mu\text{L O}_2/\text{h} \times \text{mg DW}$); reference number as in Suppl. material 1: table S2.

Taxa	E	DW	T	OCR	REF
Copepoda					
<i>Diacyclops belgicus</i>	SB	0.001	15.0	6.722	16,17
<i>Eucyclops serrulatus</i>	nSB	0.003	15.0	8.025	16
<i>Moraria</i> sp.	SB	0.001	8.0	1.122	23
Malacostraca					
<i>Asellus aquaticus</i>	nSB	2.41	11.0	1.159	4,5
<i>Asellus aquaticus carniolicus</i>	nSB	7.26	10.0	0.383	18
<i>Asellus aquaticus cavernicolus</i>	SB	4.37	10.0	0.283	18
<i>Gammarus acherondytes</i>	SB	8.93	12.9	1.311	10
<i>Gammarus fossarum</i>	nSB	4.58	11.0	0.734	4,5,8,9,11,13
<i>Gammarus lacustris</i>	nSB	1.20	12.0	14.867	6
<i>Gammarus minus</i>	nSB			2.347	1
<i>Gammarus troglophilus</i>	nSB	18.46	12.9	2.979	10
<i>Niphargus krameri</i>	SB	2.24	10.0	0.467	9
<i>Niphargus rhenorhodanensis</i>	SB	1.91	11.3	0.420	4,5,8
<i>Niphargus stygius</i>	SB	4.92	10.0	0.931	9,11,13,18,21
<i>Niphargus virei</i>	SB	13.81	11.3	0.247	4,5,12
<i>Niphargus zagrebensis</i>	nSB	4.10	11.0	1.860	18,21
<i>Proasellus lusitanicus</i>	SB	4.70	17.0	0.043	22
<i>Proasellus</i> sp. 1 (gr. <i>cavaticus</i>)	SB		9.9	0.516	15
<i>Proasellus</i> sp. 2 (gr. <i>cavaticus</i>)	SB		11.0	0.579	15
<i>Proasellus valdensis</i> (PV1)	SB		11.7	0.638	15
<i>Proasellus valdensis</i> (PV2)	SB		6.3	0.525	15
<i>Procambarus clarkii</i>	nSB	3184.50	19.0	2.770	2
<i>Procambarus franzi</i>	SB	714.00	18.0	1.500	2
<i>Procambarus pallidus</i>	SB	1737.00	19.0	0.450	2
<i>Procambarus pictus</i>	nSB	537.00	16.0	3.220	2
<i>Stenasellus virei</i>	SB	1.68	11.0	0.397	4,5
<i>Stygobromus</i> sp.	SB	1.20	7.0	6.089	4
<i>Stygobromus pecki</i>	SB	2.01	23.0	0.493	20
<i>Stygonectes emarginatus</i>	SB			1.793	1
<i>Stygonectes spinatus</i>	SB			2.653	1
<i>Stygonectes tenuis potamacus</i>	SB			2.673	1
<i>Synurella</i> sp.	nSB	1.07	23.0	4.853	20
Amphibia					
<i>Proteus anguinus</i>	SB	2625.00	12.8	0.065	7,19
Teleostei					
<i>Astyanax mexicanus</i>	SB			0.043	19
<i>Gobio occitaniae</i>	nSB			0.137	19
<i>Psalidodon fasciatus</i>	nSB	937.50	25.0	1.818	3
Actinopterygii					
<i>Poecilia mexicana</i>	nSB	38.10	25.0	2.333	14

OCRs compared to their stygobitic congeners in the genera *Asellus*, *Niphargus* and *Procambarus* (Table 1). This trend, however, was not observed in the genus *Gammarus*, where the pattern differed (Table 1). The OCRs of stygobitic cyclopoids appear to be approximately six times higher than those of stygobitic harpacticoids, both belonging to the class Copepoda (Table 1).

Body mass explained most of the linear variation in OCR ($R^2 = 0.908$). In detail, the model (Fig. 2) showed a slope (b) of 0.80 (95% CI: 0.72–0.98, SE: 0.09, $p = 0.00005$) with an intercept of -0.39 (95% CI: -0.57 – -0.21, SE: 0.07, $p < 0.00001$).

The Arrhenius plots of \ln OCR against the reciprocal of the absolute temperature are shown for the stygobitic and non-stygobitic species in Fig. 3a and Fig. 3b, respectively. The response of stygobitic species to increasing temperature followed the classic bell-shaped profile (Fig. 3a), while the lack of data for non-stygobitic species made this response less conspicuous (in 5 cases only one data point was provided for a given temperature class; Fig. 3b). The E_a for the low temperature range (from -2 to 18 °C) was 94.7 ± 20.3 kJ/mol for the stygobitic species and 132.2 ± 35.3 kJ/mol for the non-stygobitic ones. In the high temperature range (from 19 to 30 °C), the value was -232.7 ± 63.1 kJ/mol for the stygobitic species and -83.6 ± 48.1 kJ/mol for the non-stygobitic ones. The comparison of the slopes between stygobites and non-stygobites showed no statistically significant difference in the response of the OCR to temperature changes (Table 2).

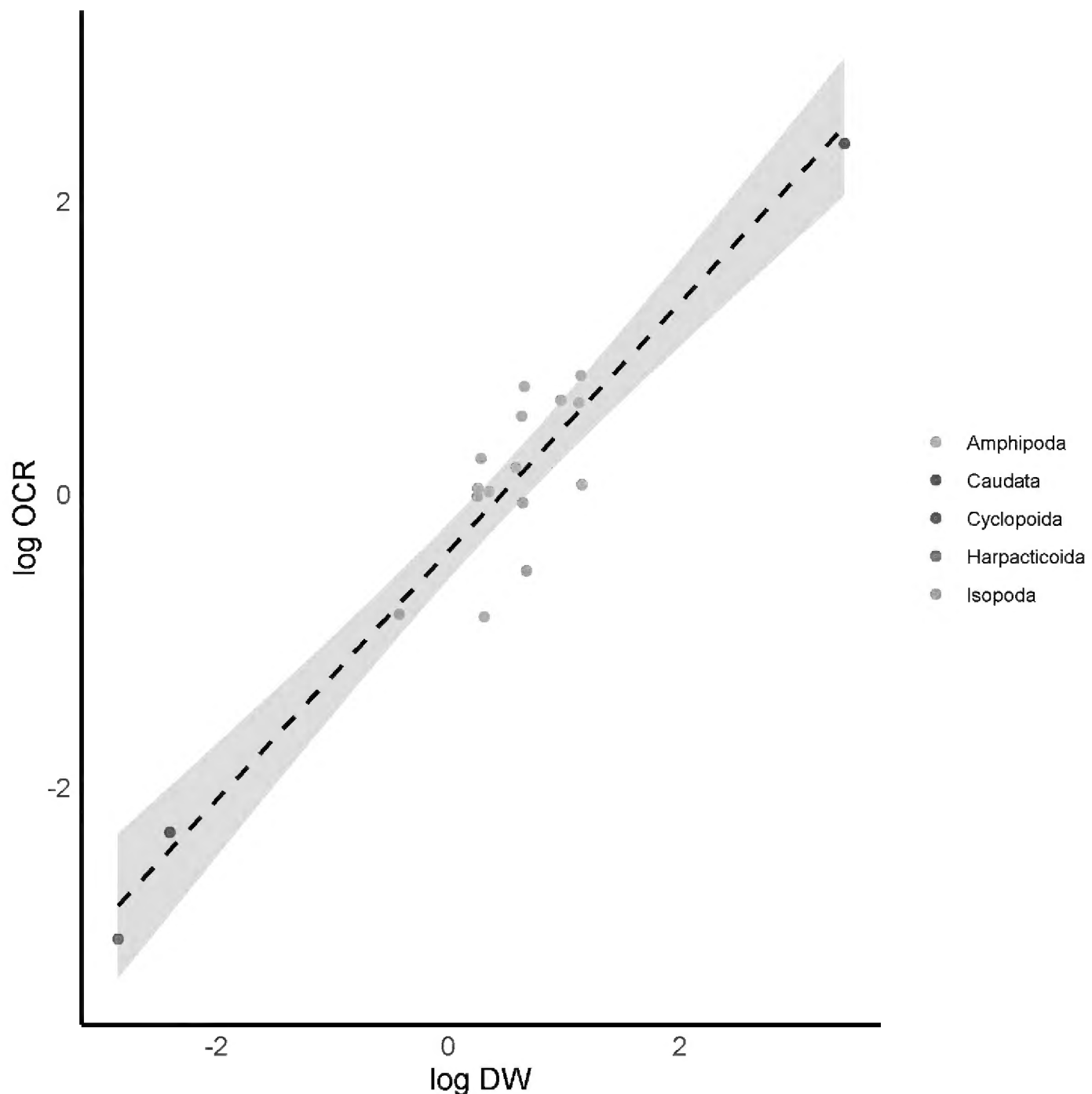


Figure 2. Oxygen consumption rates (in $\mu\text{L O}_2 / \text{ind} \times \text{h}$) as function of body weight (dry weight in mg) in stygobitic species.

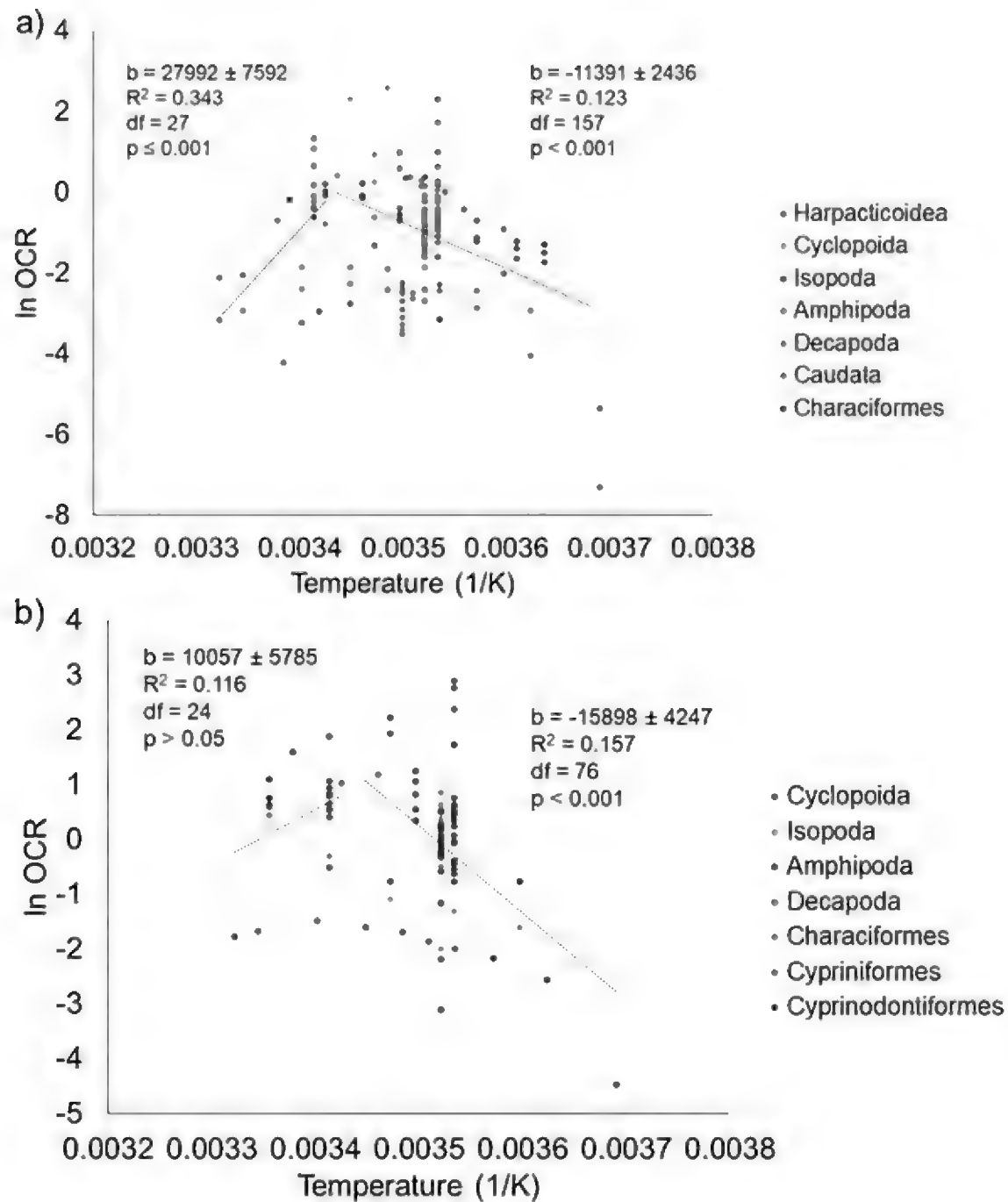


Figure 3. Arrhenius plots of oxygen consumption rates (OCR), in $\mu\text{L O}_2/\text{mg DW} \times \text{h}$ for (a) stygobitic and (b) non-stygobitic species.

Table 2. Results of ANCOVA for the comparison of the slopes between stygobites and non-stygobites (Group) using reciprocal of absolute temperature (Temp) and group as factors, analyzed for two temperature ranges.

	df	MS	F	p
Temp range -2–18 °C				
Group	1	1.296	0.889	0.347
Temp	1	44.052	30.222	<0.001
Group \times Temp	1	1.161	0.796	0.373
Error	231	1.458		
Corrected Total	234			
Temp range 19–30 °C				
Group	1	3.918	3.573	0.065
Temp	1	17.200	15.684	<0.001
Group \times Temp	1	3.722	3.394	0.071
Error	49	1.097		
Corrected total	52			

Discussion

Our analysis of oxygen consumption rates across groundwater organisms has unveiled significant insights into the metabolic physiology of both stygobitic and non-stygobitic species. This research highlighted a substantial increase in related studies since the year 2000, reflecting the escalating scientific interest in understanding the complex dynamics of groundwater fauna (Koch et al. 2024). The research efforts have been notably focused on specific taxa, such as Amphipoda, underscoring interest in the ecological and physiological adaptations of this order, further evidenced by recent research (e.g., Premate and Fiser 2024). Additionally, the investigation into the effects of temperature on OCR, especially regarding amphipods, emphasizes the significance of temperature—particularly in the context of global warming—as a pivotal environmental factor influencing metabolic rates in groundwater ecosystems (Vaccarelli et al. 2023). The focus on studying species from caves highlights the importance of these habitats in researching subterranean fauna, being caves more directly and easily accessible. Yet, the lack of stygobitic species from the hyporheic zones from this research underscores a significant gap. It would be important to address this omission, particularly given the integral connection between groundwater and surface water ecosystems (Sacco et al. 2024). The range of OCR reflects the metabolic diversity among groundwater communities. These variations are crucial for understanding the ecological roles and adaptations of these species to subterranean life. Notably, non-stygobitic species exhibited higher OCRs compared to their stygobitic counterparts in certain genera, except for *Gammarus*. This pattern indicates the presence of divergent metabolic strategies, possibly mirroring adaptations to different ecological niches and evolutionary responses to the energetically constrained subterranean habitats (Hose et al. 2022). The finding that stygobitic cyclopoids exhibit OCRs roughly six times higher than those of harpacticoids, despite both belonging to the class Copepoda, underscores considerable metabolic variability among closely related groups. This variability indicates that the metabolic rates of groundwater species are determined by a complex array of factors extending beyond temperature and body mass.

Our analyses revealed that OCR of stygobitic species scale with body weight following an allometric pattern, with a slope of 0.80. This indicates that OCR do not scale isometrically; in other words, metabolic rates do not double when the body weight doubles. This scaling pattern is consistent with findings in crustaceans (e.g., Ivlevla 1980; Glazier et al. 2010) and other surface taxa, both aquatic and terrestrial (Peters 1983; Schmidt-Nielsen 1984; Calder 1996). Several reasons underpin non-isometric scaling, encompassing heat exchanges with the environment (larger animals have a lower surface area relative to their volume, which reduces the energy required per unit of weight to maintain body temperature), energy transportation (the circulatory and respiratory systems do not scale linearly with size) and cellular and mitochondrial factors (White and Kearney 2013). If other underlying reasons remain elusive (Hoppeler and Weibel 2005), the principle of allometric scaling of metabolic rates stands as a cornerstone in the realm of biological scaling. It encapsulates the optimization of energy

utilization across a spectrum of organism sizes, including those inhabiting groundwater environments, which appear to adhere to this universal pattern without deviation.

A significant portion of the intra-specific metabolic rates' variation can also be explained by differences in the mass. Shokri et al. (2019) demonstrated that individual metabolic rates scale allometrically with body mass in three species of surface amphipods. However, the intra-specific slopes were in the range of 0.32–0.36. This indicates a moderate mass-dependent variation of metabolic rates within an individual species, which can allow it to broaden its ecological niche, contributing to greater functional diversity. Noteworthy, OCR appears to be mass-independent in certain stygobitic species (Hose et al. 2022), as observed in studies on *D. belgicus* (Di Lorenzo et al. 2015), *P. lusitanicus* (Di Lorenzo and Reboleira 2022) and *G. acherondytes* (Wilhelm et al. 2006). This pattern (described as ametric in Di Lorenzo et al. 2015) has been regarded as an adaptive trait to energy-limited groundwater habitats, where individuals may increase in size without increasing in their metabolic rates. This phenomenon likely occurs because weight gain is attributed to metabolically inactive reserves (stored fats, water, or other substances that add to the weight but do not participate in respiration) as body mass increases. Stygobitic species possess larger fat reserves compared to their surface-dwelling relatives (Hüppop 1986; Culver et al. 1995). This trait allows stygobitic species to endure prolonged periods without food by relying on stored energy (Hervant et al. 1997), thereby enhancing their resilience to the challenges posed by their habitats (Di Lorenzo et al. 2023 and references therein). On the other hand, the low metabolic variability among individuals of the same species may heighten population vulnerability and diminish the resilience to deviations from the norm, such as those stemming from anthropogenic disturbances (Hose et al. 2022). Further studies are necessary to confirm whether this trait is shared among stygobitic species or not.

The Arrhenius activation plots, which illustrate the effect of temperature on organisms over the range -2 to 30 °C, showed a temperature break in the OCR for both stygobitic and non-stygobitic species that were included in our study according to the procedure described in the Methods. Lower E_a values observed in stygobitic species compared to non-stygobitic ones within the low temperature range (-2–18 °C) indicate that the OCR of stygobitic species increase less with increasing temperature. This implies that the metabolic rates of stygobitic species are less responsive to temperature changes than that of non-stygobitic species. These findings align with previous research examining the response of potential metabolic activity, determined as a proxy for cellular respiration, to temperature changes in *N. stygius* (Simčič and Sket 2019). The reason for the relatively stable metabolic potential could be the reduction in costs for mitochondrial biosynthesis and degradation. The observed pattern could be also related to the low resource availability in subterranean habitats. Furthermore, the cost of maintaining a higher number of mitochondria, e.g. maintaining proton gradients and aerobic enzyme capacities, would contribute to an increase in basal metabolic rate (Rolfe et al. 1999; Pörtner 2002). As a result, this increase would lead to a shift in the energy

balance, which would have an unfavorable effect on the accumulation of energy reserves for growth and reproduction (Lannig et al. 2003). Biochemical indicators of metabolic activity are key components of biochemical metabolic pathways that are directly or indirectly linked to processes important for performance, growth and/or reproduction. The basic premise for their use is that adjustments in the rates of physiological processes are necessary to balance metabolic demand with available energy supply (Dahlhoff 2004).

The E_a values for stygobitic species within -2 to $18\text{ }^{\circ}\text{C}$ ($94.7 \pm 20.3\text{ kJ/mol}$) are in the range of values reported by Mermillod-Blondin et al. (2013) for three isopod species of the genus *Proasellus* that colonize groundwater habitats with stable temperatures (annual temperature amplitude $<1\text{ }^{\circ}\text{C}$) and by Simčič and Sket (2019) for the hypogean population of *A. aquaticus cavernicolus*. However, the E_a values in this study (0.98 eV and 1.3 eV for -2 to $18\text{ }^{\circ}\text{C}$ and -2.41 and -0.86 eV for 18 to $30\text{ }^{\circ}\text{C}$, stygobitic and non-stygobitic species), exceed the average 0.48 eV reported for 314 aquatic and terrestrial ectothermic species (fishes, crustaceans, mollusks, amphibians and insects) within their permissive temperature range (0 to $40\text{ }^{\circ}\text{C}$; Jorgensen et al. 2022). This suggests that the temperature ranges we investigated in this study might not entirely fall within the permissive thermal range for the species analyzed. The high E_a values observed in our study implies that climate change could escalate heat-related energy expenditure for stygobitic and non-stygobitic species, with potential severe outcomes, as observed in many other ectothermic species (Jorgensen et al. 2022). Our findings suggest that even modest global warming scenarios may impose greater metabolic impairments on stygobitic species compared to non-stygobitic ones, in line with results of a recent meta-analysis on the effects of climate change on subterranean ecosystems (Vaccarelli et al. 2023). In fact, in the high temperature range (18 – $30\text{ }^{\circ}\text{C}$), the Arrhenius plot showed faster decline in OCR in stygobitic species than in non-stygobitic species. This indicates that hypogean species have a less efficient enzyme system at higher temperatures than epigean species. This might be due to the inactivation of respiratory enzymes that limit enzymatic processes outside their usual temperature range, leading to an irreversible loss of functions (Yurista 1999), as thermal tolerance is subject to phenotypic alteration within genetically defined limits (Cuculescu et al. 1998). Enzyme structure and function are highly sensitive to temperature variations, whether these changes occur rapidly or over evolutionary timescales (Angilletta 2009). The adaptive responses to these stresses have played a significant role in biological evolution (Clarke 2017). Studies have demonstrated that temperature impacts on enzyme properties establish the thermal optima and tolerance limits of metabolic functions, which are crucial for the survival and performance of organisms (Angilletta 2009). It appears that stygobitic species are less capable of maintaining optimal activity at higher temperatures, indicating a greater sensitivity to elevated temperatures compared to non-stygobitic species. The discrepancy indicates that surface species may possess a more effective molecular defense mechanism against thermal stress compared to subterranean counterparts. Most probably due to the fact that non-stygobitic spe-

cies live in environments with high fluctuations in water temperatures. Previous studies have shown that there are variations in the genes associated with responses to thermal stress between subterranean and surface-dwelling terrestrial species, with a notably larger number of genes showing differential expression in surface taxa, suggesting a more robust heat shock response capability (Beasley-Hall et al. 2022). Although this has not yet been specifically demonstrated for aquatic subterranean species, it carries implications regarding the reduced thermal tolerance of stygobitic organisms at the molecular level.

Our review highlighted that the studies aiming towards understanding of the influence of temperature on metabolic rates of stygobitic species remain disconnected from broader ecological considerations such as energy budgets, food web dynamics and ecosystem functioning. Metabolic heat production and loss, critical in energy transfer efficiency between trophic levels, are yet to be fully integrated into these studies. The reports of such effects on metabolic scaling usually focus on single factors, such as comparison in metabolism between stygobitic and non-stygobitic species, the investigation of different species responses under different temperature acclimation and under different experimental set-ups (hypoxia/normoxia; starvation, exposure to pollutants) or investigation of metabolic rates of species from different localities or habitats, while understanding the possibility of significant interactive effects between multiple factors requires further studies.

This study provides insights in the metabolism of groundwater species, though there are potential limitations and areas that could benefit from further research. Extracting relevant data for our study was hindered by the inconsistent reporting of experiments, which frequently omitted detailed information on body size and weight. We bridged these gaps with calculations and estimations. Nonetheless, the assumption made on computation of the dry weight and the inclusion in the dataset of species that have not yet been described represent a limitation of our study. We also acknowledge potential limitations of our approach aimed at assessing the effect of temperature on the metabolic rates of groundwater species. Pooling data from different species into broad groups (stygobitic and non-stygobitic) might mask species-specific variations, potentially leading to an oversimplified interpretation of the data. Additionally, certain species are overrepresented in specific temperature classes, leading to a potential uneven comparison between the two ecological groups. The potential bias toward frequently studied species and regions signals the direction toward studies should expand in the future. Additionally, while this study explores the influence of environmental factors, such as temperature on OCR, more research is needed to understand the metabolic strategies of stygobitic species fully. Future research should focus on lesser-studied habitats, including hyporheic zones and employ experimental approaches to assess environmental stressors on metabolic rates. Integrating energy budgets, food web dynamics and ecosystem functioning into metabolic studies will provide a deeper understanding of groundwater species' resilience and inform conservation strategies against anthropogenic impacts and climate change.

Conclusion

Our investigation inquired into the metabolic rates of groundwater organisms, focusing on the relationship between oxygen consumption rates (OCR), body weight and temperature. We uncovered an allometric scaling of OCR with body weight and revealed a distinct thermal sensitivity between stygobitic and non-stygobitic species, underscoring a nuanced vulnerability to changing temperatures. However, the reliance on existing literature may introduce biases towards more frequently studied species and regions, potentially skewing the universality of our conclusions. Moreover, the varied methodological approaches across studies present challenges in data standardization, emphasizing the need for uniform reporting standards in future metabolic rate research. The implications of our findings are potentially far-reaching, providing insights for the conservation and management of groundwater ecosystems, particularly in the face of global climate change. For example, understanding the metabolic responses of stygobitic species to temperature changes can inform conservation strategies to protect these species in regions predicted to experience significant warming (Shokri et al. 2022). However, to build upon our work, research should expand to include lesser-studied groundwater habitats and employing experimental approaches to directly assess the impact of environmental stressors on metabolic rates. Such endeavors would enrich our comprehension of subterranean life's resilience and adaptability, informing strategies to safeguard these vital ecosystems against anthropogenic impacts and climatic shifts.

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Supplementary material I

Supplementary information

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Data type: docx

Explanation note: **table S1.** Oxygen consumption rates ($\mu\text{L O}_2/\text{h} \times \text{mg DW}$) for stygobitic and non-stygobitic species divided into 11 temperature classes (TC) with an interval of 3 degrees, from -2°C to 30°C . N: number of measurements. **table S2.** List of literature sources with geographic information and aims of the studies. **fig. S1.** Quantile-Quantile (QQ) plot assessing the normality of the residuals from the generalized linear model with data not corrected by temperature (Shapiro test: $W = 0.928$, $p\text{-value} = 0.180$).

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